

WHAT IS CLAIMED IS:

1. An isolated and purified ATP diphosphohydrolase obtainable from bovine aorta characterized by the following physico-chemical properties:

5 -a catalytic unit of a molecular weight on denaturing polyacrylamide gel electrophoresis of about 78 KDa in its native form;

10 -a deglycosylated form of said catalytic unit of a molecular weight on SDS-PAGE of about 56 KDa; and characterized in that it comprises the amino acid sequences defined in SEQ. ID. NOS. 3 to 6.

2. An ATP diphosphohydrolase as defined in claim 1 further comprising the amino acid sequence defined in SEQ. ID. No.: 8 or a variant thereof.

15 3. An isolated and purified ATP diphosphohydrolase obtainable from pig pancreatic zymogen granules

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characterized by the following physico-chemical properties:

-a catalytic unit of a molecular weight on denaturing polyacrylamide gel electrophoresis of about
5 54 KDa in its native form;

-a deglycosylated form of said catalytic unit of a molecular weight on SDS-PAGE of about 35 KDa; and characterized in that it comprises the amino acid sequence defined in SEQ. ID. NO.: 7.

10 4. A process for purifying an ATP-diphosphohydrolase enzyme from a tissue capable to convert ATP to ADP and ADP to AMP which comprises:

a) obtaining a sub-cellular microsomal fraction from an homogenate of said tissue;

15 b) solubilizing said microsomal fraction in the presence of a non-ionic detergent;

c) centrifuging said solubilized microsomal fraction to obtain a supernatant containing said enzyme;

d) submitting said supernatant to an ion-exchange chromatography to obtain a first enzyme eluate;

e) submitting said first eluate to an affinity column chromatography to obtain a second enzyme eluate;

5 and

f) submitting said second eluate to a separation step on a non-denaturing gel electrophoresis to recover said enzyme free of any contaminant, the presence of said contaminant being monitored by overstaining said gel in a silver nitrate dye or Coomassie Blue dye.

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5. A process according to claim 4 wherein said ion exchange chromatography is achieved on a column containing Diethylaminoethyl (DEAE).

6. A process according to claim 5 wherein said column is a DEAE agarose column.

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7. A process according to claim 4 or 5 wherein said affinity column chromatography is achieved on an Affigel™ Blue column.

5 8. A process according to claim 4, 5, 6 or 7 wherein said non-ionic detergent is Triton X-100™.

9. A process according to claim 4, 5, 6, 7 or 8 wherein an aliquot of said enzyme is further submitted after step f) to a polyacrylamide gel electrophoresis under denaturing conditions to verify its homogeneity and to
10 obtain its apparent molecular weight.

10. A process according to claim 9 wherein said enzyme is obtained from pig pancreatic zymogen granules and has an apparent molecular weight of 54 Kilodaltons.

11. A process according to claim 9 wherein said enzyme
15 is obtained from bovine aortic intima layer and has an apparent molecular weight of about 78 Kilodaltons.

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12. A process according to claim 10 wherein, between steps e) and f), a step of deglycosylation is included, and whereby the apparent molecular weight is shifted from 54 to 35 KDa.

5 13. A process according to claim 11 herein, between steps e) and f), a step of deglycosylation is included, and whereby the apparent molecular weight is shifted from 78 to 56 KDa.

10 14. The use of the ATP diphosphohydrolase of claim 1 or 2, for reducing platelet aggregation and thrombogenicity.

15 15. The use of an ATP diphosphohydrolase for reducing platelet aggregation and thrombogenicity, said ATP diphosphohydrolase having the amino acid sequence defined in SEQ. ID. NO.: 1, or a variant thereof, or a part thereof, said variant or part being capable of converting ATP to ADP and ADP to AMP.

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16. A composition for use in the reduction of platelet aggregation and thrombogenicity which comprises as an active ingredient the ATP diphosphohydrolase of claim 1 or 2 or an ATP diphosphohydrolase which sequence is defined in SEQ. ID. NO.: 1, or a variant or a part thereof, which variant or part has an ATP diphosphohydrolase activity, in an acceptable pharmaceutical carrier.

17. A process for producing an ATP diphosphohydrolase which comprises the steps of:

- obtaining a host which comprises a nucleic acid encoding a protein having the amino acid sequence defined in SEQ. ID. NO.: 1, or a variant thereof, or a part thereof, said variant or part being capable of converting ATP to ADP and ADP to AMP;

- culturing said host in a culture medium supporting the growth of said host and the expression of said nucleic acid;

- recovering the ATP diphosphohydrolase from the culture medium or from said host; and
- purifying the ATP diphosphohydrolase.

18. A process as defined in claim 17, wherein said
5 nucleic acid has a sequence defined in SEQ. ID. NO.: 2,
a variant thereof or a part thereof, said variant or
part being capable of producing an ATP
diphosphohydrolase which converts ATP to ADP and ADP to
AMD.

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